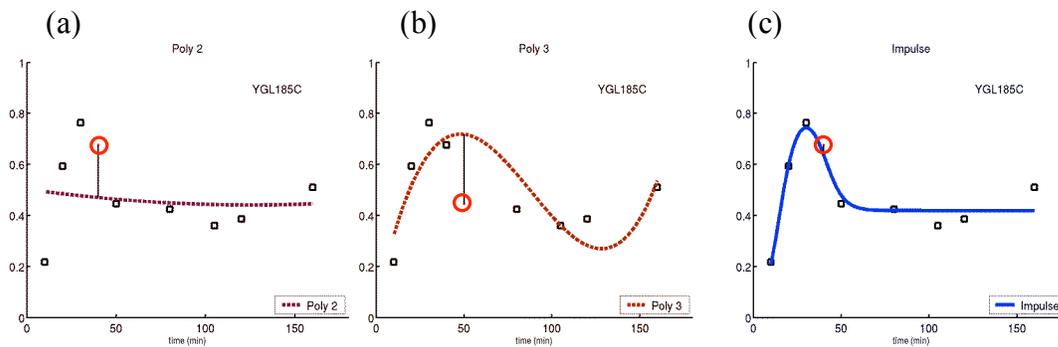


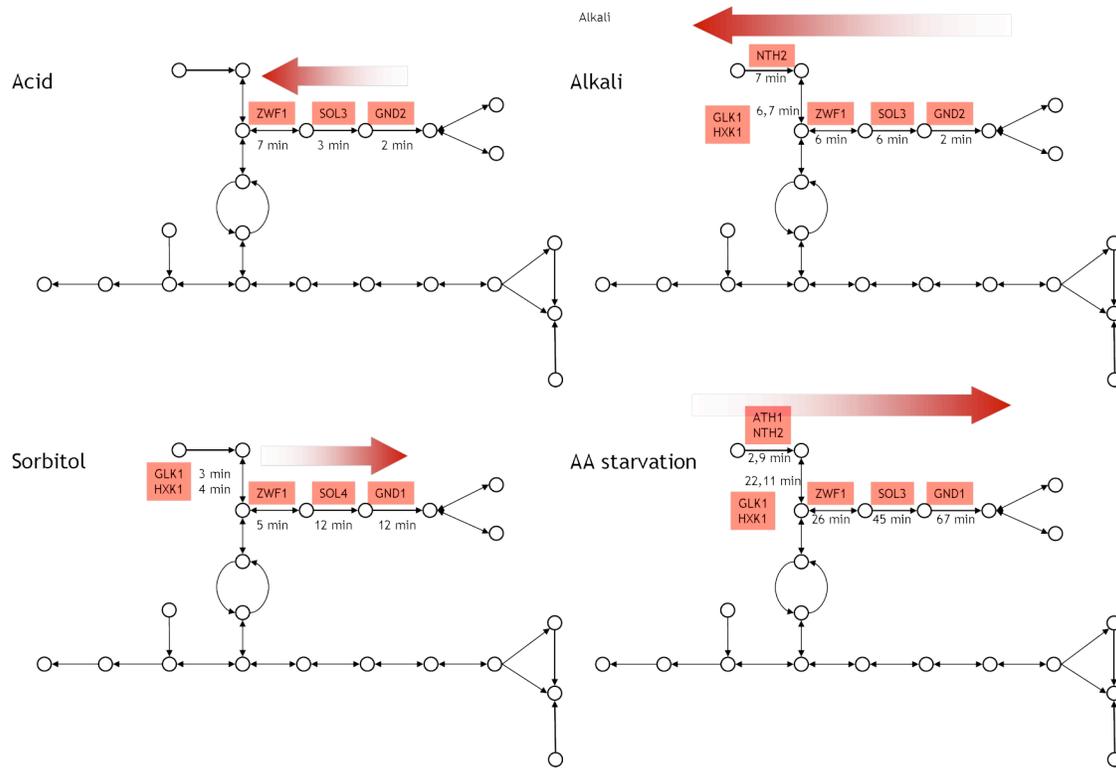
## Supplemental figures and tables

Figure S1: An *impulse* model for gene expression time courses.



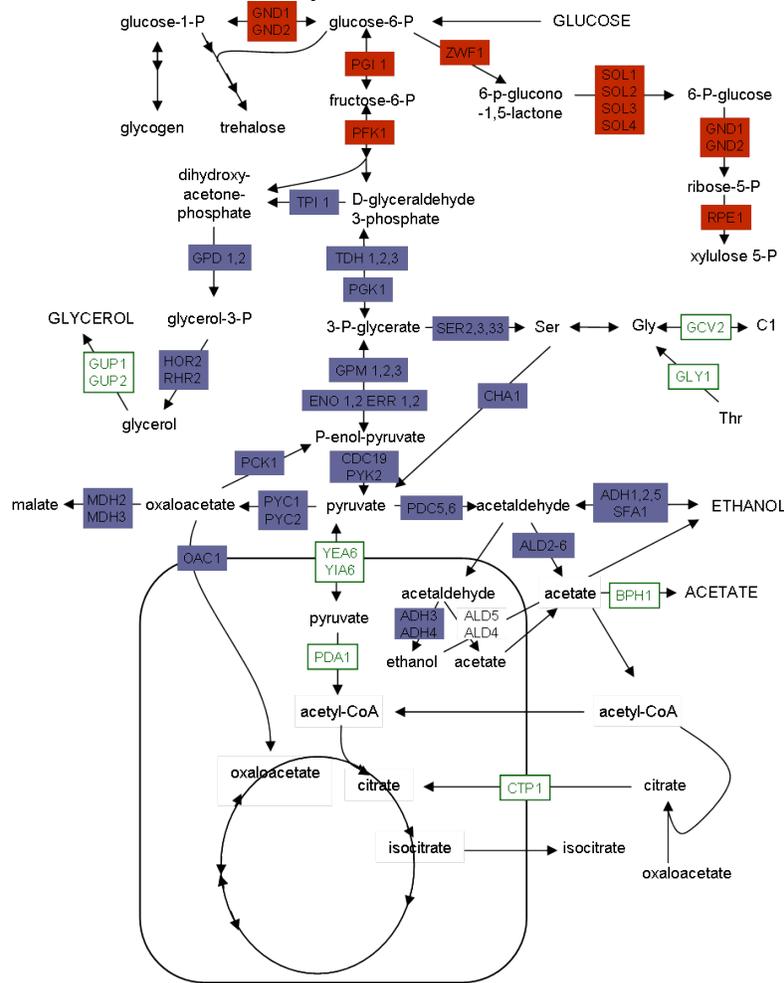
**Fitting an expression profile of a single gene using “leave-one-out”.** Black squares denote measurements that were used for the fit, red dot denotes a single measurement that was hidden, and the vertical black line denotes the prediction error for the hidden measurement. **(a)** A 2<sup>nd</sup> order polynomial strongly under-fits. **(b)** A 3<sup>rd</sup> order polynomial mildly overfits. **(c)** The impulse model captures well the shape of this time course, since it is flexible to fit time courses with two transitions. By focusing on the relevant subspace of common expression responses to changes, it avoids overfitting, even in face of relatively low number of measurements.

Figure S2: **Timing activity motifs across additional conditions**



**Activity motif from glucose to ribulose 5-phosphate production covering the oxidative branch of the pentose phosphate pathway (PPP). Backward-activation in acid (a) and alkali (b). Forward-activation in sorbitol (c) and amino acid starvation (d). See Fig. 3 in the main text for details.**

Figure S3: **Distribution of timed enzymes across metabolism.**



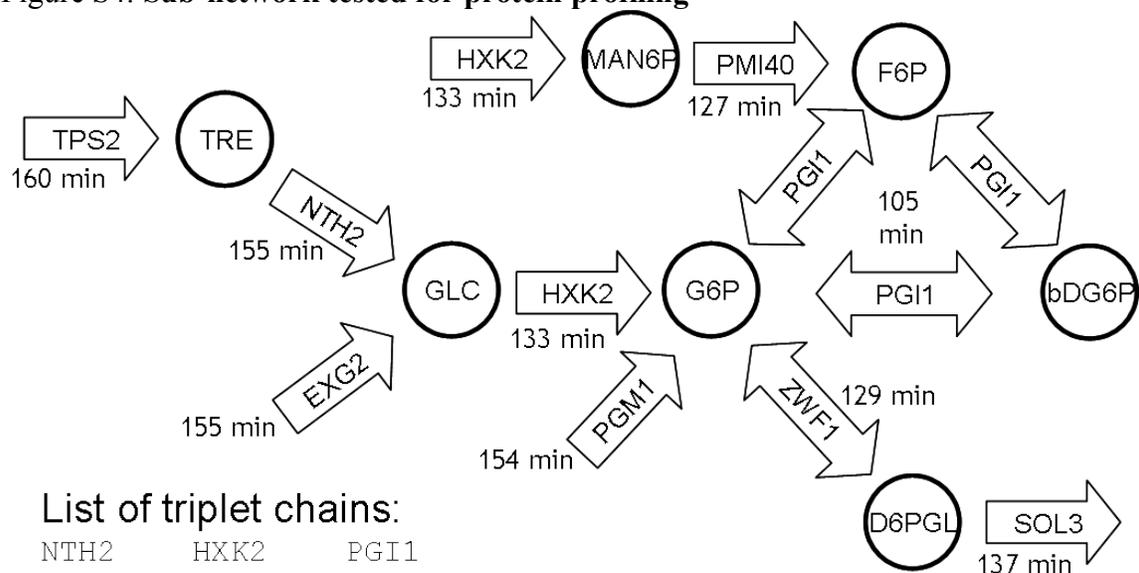
**Clusters of timing-activity-motifs.** TAMs were clustered by aggregating together chains of length 4 that had at least two common reactions. Timed enzymes naturally cluster into two groups (red, blue), together covering most of, and almost only, central carbon metabolism.

The full list of enzymes in each of the two components (bold enzymes correspond to entries in the above figure) is:

Component 1: CDC19 ADH5 **PYC2** MAL31 AGP1 CHA1 PGK1 BPH1 GPM2 GPD1 MDH3 SFA1 TPI1 YDR111C GNP1 HOR2 SER3 DAK2 AGP3 PYC1 ADH4 PDC6 TDH3 SER2 ENO1 ENO2 RHR2 SER33 YIL167W TDH1 TDH2 OAC1 GPM1 JEN1 GAP1 PCK1 FPS1 AAT2 PDC1 PDC5 ADE13 DAK1 ALD3 ALD2 ADH2 YMR323W GPM3 GPD2 ADH1 MDH2 SER1 PYK2 **ERR1** ALD6 FUM1 DIP5 ERR2

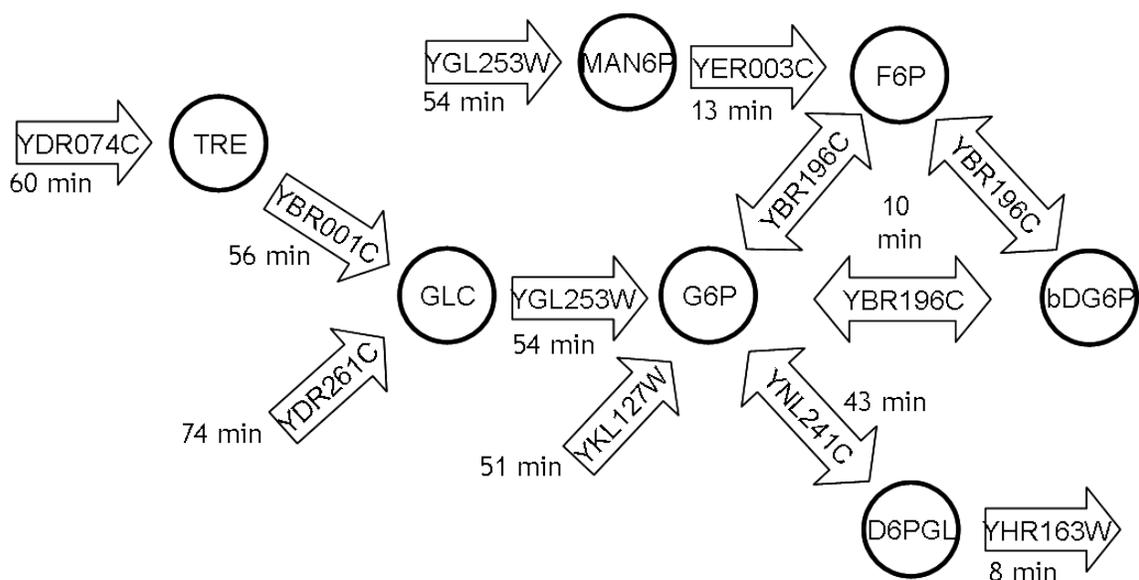
Component 2: PRS4 NTH2 **PGI1** MAL32 GLK1 YCR073W\_A HXT15 NTH1 TPS2 EXG2 HXT7 HXT6 HXT3 STL1 GLC3 HXT13 PMI40 HIS1 PRS2 HXT10 SEC53 HXK1 HXK2 **PFK1 SOL4 GND2** BGL2 YGR287C MAL12 PRS3 YHR046C HXT4 HXT1 HXT5 **SOL3 GND1** PFK26 YIL172C **RPE1** INO1 FBP26 HXT8 YJL216C HXT9 FSP2 HXT16 SOR1 FBA1 PGM1 PRS1 GAL2 EXG1 FBP1 HXT2 PGM2 PFK2 **ZWF1 SOL1** HXT17 PRS5 PFK27 HXT11 RKI1 SPR1 ATH1 GPH1

Figure S4: **Sub-network tested for protein profiling**



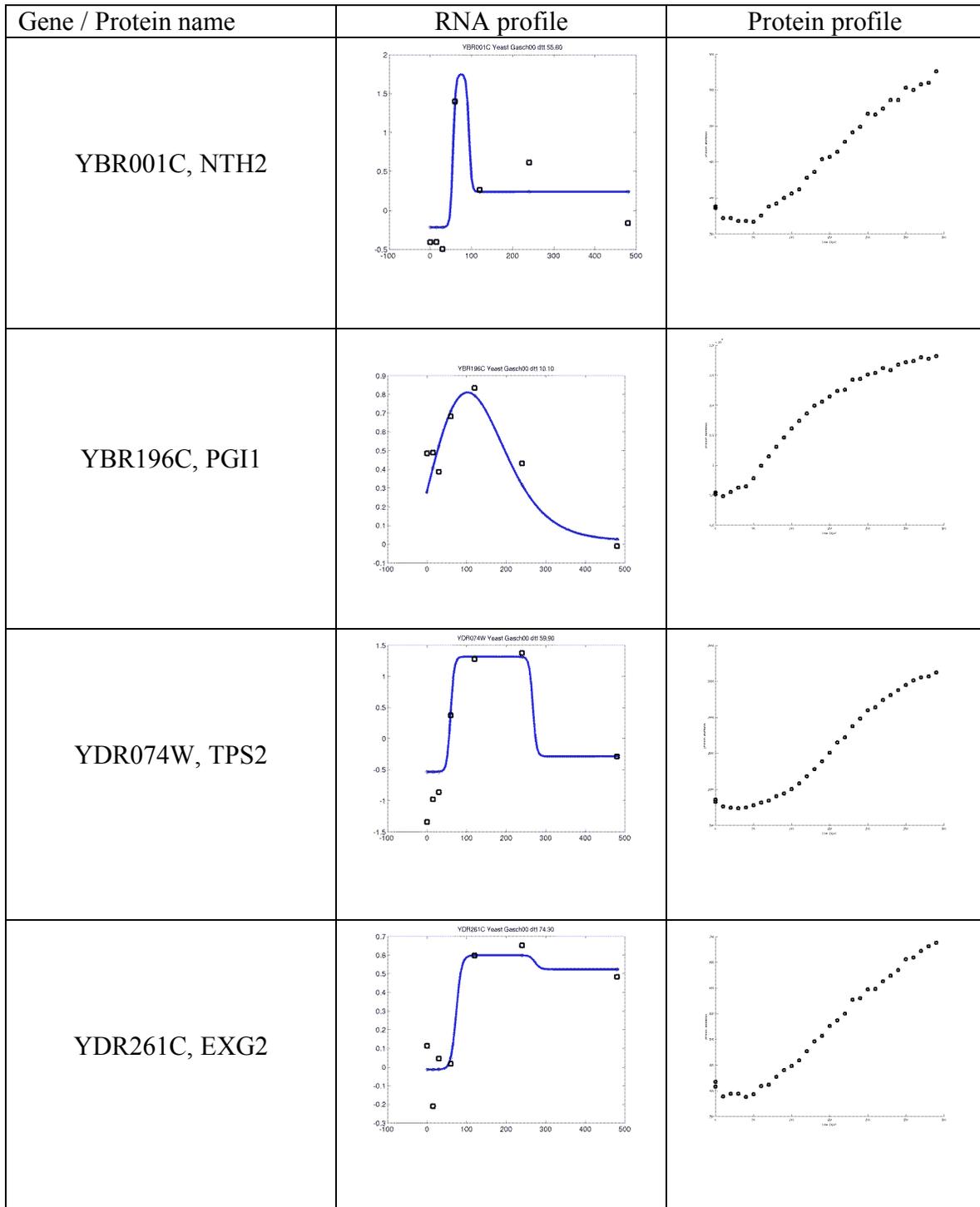
List of triplet chains:

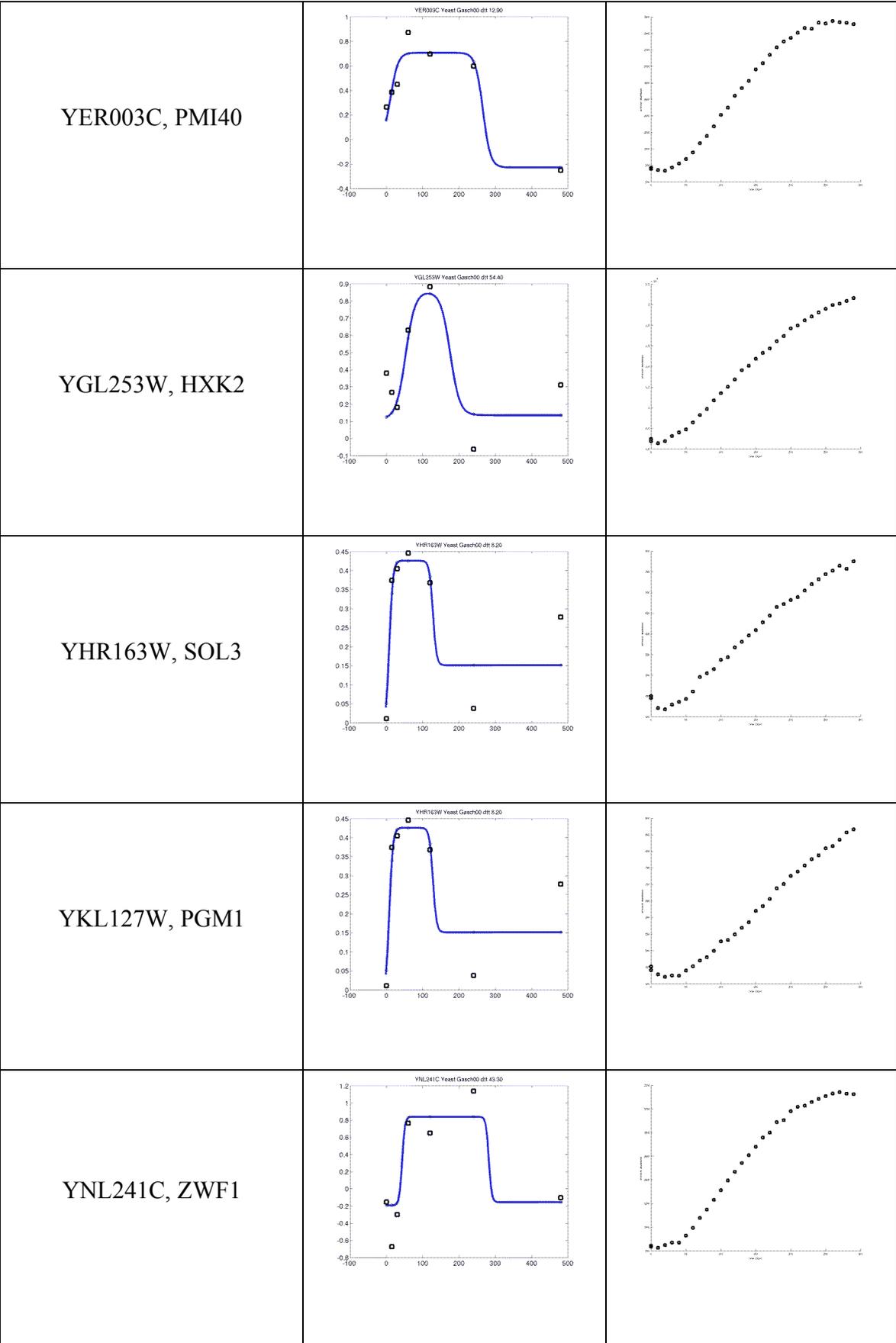
|      |       |      |
|------|-------|------|
| NTH2 | HXK2  | PGI1 |
| NTH2 | HXK2  | ZWF1 |
| TPS2 | NTH2  | HXK2 |
| EXG2 | HXK2  | PGI1 |
| EXG2 | HXK2  | ZWF1 |
| HXK2 | PMI40 | PGI1 |
| HXK2 | ZWF1  | SOLW |
| PGM1 | ZWF1  | SOLW |



**Top:** Network of the 9 proteins tested for responses to DTT. Timing numbers are the average onset over two replicates. Except for motifs that include SOL3, all triplets follow a *backward-activation* TAM. **Bottom:** same network, annotated with RNA onset times.

Figure S5: RNA profile and corresponding Protein profiles





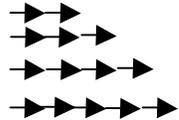
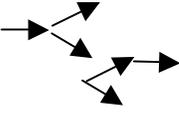
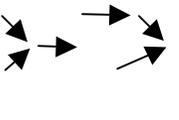
## References

1. Voit, E. O. Biochemical and genomic regulation of the trehalose cycle in yeast: review of observations and canonical model analysis. *J Theor Biol* **223**, 55-78 (2003).
2. Voit, E. O. Utility of Biochemical Systems Theory for the analysis of metabolic effects from low-dose chemical exposure. *Risk Anal* **20**, 393-402 (2000).

**Table S1: List of network motifs and activity motifs that we tested.**

The set of wiring motifs consists of all types of network sub-graphs consisting of 2 or 3 enzymes, as well as longer chains. For each wiring pattern, the set of activity patterns is created by combining two features for each enzyme: (1) timing order pattern, and (2) activation/repression. The timing patterns for each network motif contain all onset configurations that are strongly ordered (for a chain relation) or weakly ordered (for forks and joins) (see **Methods**). The activation/repression pattern required that all genes are either activated or repressed.

For example, for a chain sub-graph, four activity motifs are defined, created by combining one of two options for On/Off and on of two options for forward/backward.

| Name         | size             | Wiring patterns   | Activity patterns   |    |
|--------------|------------------|---|---|----|
| Chain        | 2<br>3<br>4<br>5 |    | <b>{Forward, Backward} x {OnOff, OffOn}</b><br>ForwardOnOff, BackwardOnOff,<br>ForwardOffOn, BackwardOffOn                      | 16 |
| Fork-2       | 2                |    | <b>Same Time x {OnOff,OffOn}</b><br>SameOnOff, SameOffOn  | 2  |
| Fork-3       | 3                |  | <b>{All Same Time, Forward+Same, Backward+Same} x {OnOff,OffOn}</b><br>SameFwdOnOff, SameFwdOffOn<br>SameBwdOnOff, SameBwdOffOn | 8  |
| Multi-Fork   | 3                |  | <b>All Same Time x {OnOff,OffOn}</b><br>SameOnOff, SameOffOn  | 2  |
| Funnel-2     | 2                |  | <b>Same Time x {OnOff,OffOn}</b><br>SameOnOff, SameOffOn  | 2  |
| Funnel-3     | 3                |  | <b>{All Same Time, Forward+Same, Backward+Same} x {OnOff,OffOn}</b><br>SameFwdOnOff, SameFwdOffOn<br>SameBwdOnOff, SameBwdOffOn | 8  |
| Multi-Funnel | 3                |  | <b>All Same Time x {OnOff,OffOn}</b><br>SameOnOff, SameOffOn  | 2  |
|              |                  |   |   | 40 |

**Table S2: List of data sources and time courses**

| Time course  | # samples |
|--|-----------|
| Gasch00 1M sorbitol                                    | 7         |
| Gasch00 1.5 mM diamide                                 | 9         |
| Gasch00 1 mM Menadione                                 | 10        |
| Gasch00 25C  | 6         |
| Gasch00 2.5mM DTT                                      | 9         |
| Gasch00 Amino acid adenine starvation                  | 6         |
| Gasch00 Hypo osmotic shock                             | 6         |
| Gasch00 Nitrogen Depletion                             | 10        |
| Gasch00 constant 0.32 mM H <sub>2</sub> O <sub>2</sub> | 11        |
| Gasch00 dtt  | 8         |
| Gasch00 heat shock                                     | 8         |
| Gasch01 DES459 mec1 0.02 MMS                           | 8         |
| Gasch01 DES460 0.02 MMS                                | 7         |
| Gasch01 mec1 plus gamma                                | 9         |
| Gasch01 wt plus gamma                                  | 9         |
| DeRisi97 diauxic shift                                 | 6         |
| New Data SD  | 6         |
| New Data SD+aa   | 6         |
| New Data SD+aaAR+ino                                   | 6         |
| New Data SD+aaLI+ino                                   | 6         |
| New Data SD+ino  | 6         |
| New Data SEtOH   | 6         |
| New Data SEtOH+aa                                      | 6         |
| New Data SEtOH+aa+ino                                  | 6         |
| New Data SEtOH+ino                                     | 6         |
| New Data Sgal  | 6         |
| New Data Sgal+aa                                       | 6         |
| New Data Sgal+aa+ino                                   | 6         |
| New Data Sgal+ino                                      | 6         |
| Causton01 Acid   | 7         |
| Causton01 Alkali                                       | 7         |
| Causton01 Heat   | 6         |
| Causton01 NaCl   | 6         |
| Causton01 Peroxide                                     | 6         |
| Causton01 Sorbitol                                     | 6         |
| ORourke03 ZT WT 0.0625M KCl                            | 5         |
| ORourke03 ZT WT 0.125M KCl                             | 5         |
| ORourke03 ZT WT 0.25M KCl                              | 5         |
| ORourke03 ZT WT 0.5M KCl                               | 10        |
| ORourke03 ZT WT 1M KCl                                 | 9         |

|                                    |    |
|------------------------------------|----|
| ORourke03 ZT WT 1Msorb             | 10 |
| ORourke03 ZT WT alpha              | 5  |
| ORourke03 ZT hog1 0.125M KCl       | 5  |
| ORourke03 ZT hog1 0.5M KCl         | 10 |
| ORourke03 ZT pbs2 0.5M KCl         | 5  |
| ORourke03 ZT sho1 0.5M KCl         | 5  |
| ORourke03 ZT ssk1 0.0625M KCl      | 5  |
| ORourke03 ZT ssk1 0.125M KCl       | 5  |
| ORourke03 ZT ssk1 0.5M KCl         | 5  |
| ORourke03 ZT ssk1sho1 0.5M KCl     | 5  |
| ORourke03 ZT ssk1ste11 0.0625M KCl | 5  |
| ORourke03 ZT ssk1ste11 0.125M KCl  | 5  |
| ORourke03 ZT ssk1ste11 0.25M KCl   | 5  |
| ORourke03 ZT ssk1ste11 0.5M KCl    | 5  |
| ORourke03 ZT ssk1ste11 1M KCl      | 5  |
| ORourke03 ZT ste11 0.0625M KCl     | 5  |
| ORourke03 ZT ste11 0.125M KCl      | 5  |
| ORourke03 ZT ste11 0.5M KCl        | 5  |
| Lai05 WT Gal N2 R1                 | 5  |
| Lai05 WT Gal N2 R2                 | 5  |
| Lai05 WT Gal N2 R3                 | 5  |
| Lai05 WT Glu N2 R1                 | 5  |
| Lai05 WT Glu N2 R2                 | 5  |
| Lai05 WT Glu N2 R3                 | 5  |
| Lai05 msn2/4 Galactose R1          | 5  |
| Lai05 msn2/4 Galactose R2          | 5  |
| Lai05 msn2/4 Galactose R3          | 5  |
| LaiMean05 WT Gal N2 R1             | 5  |
| LaiMean05 WT Glu N2 R1             | 5  |
| LaiMean05 msn2/4 Galactose R1      | 5  |
| Zakrzewska05                       | 6  |
| Mercier05 18733 200GY              | 7  |
| Mercier05 18734 200Gy              | 7  |
| Mercier05 18735 200Gy              | 7  |
| Mercier05 6053 200Gy               | 7  |
| Mercier05 hdf1 LM79 200Gy          | 7  |

| Key           | reference   |
|---------------|---|
| Gasch 00      | Audrey P. Gasch, Paul T. Spellman, Camilla M. Kao, Orna Carmel-Harel, Michael B. Eisen, Gisela Storz, David Botstein, and Patrick O. Brown, Genomic Expression Programs in the Response of Yeast Cells to Environmental Changes, (2000) Mol. Biol. Cell. 11 (12) 4241-4257  |
| Gasch 01      | Audrey P. Gasch, Mingxia Huang, Sandra Metzner, David Botstein, Stephen J. Elledge, and Patrick O. Brown, Genomic Expression Responses to DNA-damaging Agents and the Regulatory Role of the Yeast ATR Homolog Mec1pn (2001) Mol. Biol. Cell 12 (10)  |
| ORourke 03    | Sean M. O'Rourke and Ira Herskowitz, A Third Osmosensing Branch in <i>Saccharomyces cerevisiae</i> Requires the Msb2 Protein and Functions in Parallel with the Sho1 Branch Molecular and Cellular Biology, July 2002, p. 4739-4749, Vol. 22, No. 13  |
| DeRisi 97     | Joseph L. DeRisi, et al Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic Scale, Science 278 (5338), 680 (1997)   |
| Lai 05        | Liang-Chuan Lai, Alexander L. Kosorukoff, Patricia V. Burke and Kurt E. Kwast, Dynamical Remodeling of the Transcriptome during Short-Term Anaerobiosis in <i>Saccharomyces cerevisiae</i> : Differential Response and Role of Msn2 and/or Msn4 and Other Factors in Galactose and Glucose Media, Molecular and Cellular Biology, May 2005, p. 4075-4091, Vol. 25, No. 10 |
| Causton 01    | Helen C. Causton, Bing Ren, Sang Seok Koh, Christopher T. Harbison, Elenita Kanin, Ezra G. Jennings, Tong Ihn Lee, Heather L. True, Eric S. Lander, and Richard A. Young Remodeling of Yeast Genome Expression in Response to Environmental Changes, Mol Biol Cell. 2001 February; 12(2): 323-337.  |
| Kitagawa 05   | Emiko Kitagawa, Kuniko Akama and Hitoshi Iwahashi, Effects of Iodine on Global Gene Expression in <i>Saccharomyces cerevisiae</i> , Bioscience, Biotechnology, and Biochemistry Vol. 69 (2005) , No. 12 pp.2285-2293  |
| Mercier 05    | G. Mercier, N. Berthault, N. Touleimat, F. K.p.s, G. Fourel, E. Gilson and M. Dutreix, A haploid-specific transcriptional response to irradiation in <i>Saccharomyces cerevisiae</i> , Nucleic Acids Research 2005 33(20):6635-6643   |
| Zakrzewska 05 | Anna Zakrzewska, Andre Boorsma, Stanley Brul, Klaas J. Hellingwerf, and Frans M. Klis Transcriptional Response of <i>Saccharomyces cerevisiae</i> to the Plasma Membrane-Perturbing Compound Chitosan, Eukaryotic Cell, April 2005, p. 703-715, Vol. 4,   |

Table S3: Enrichment of motifs across conditions  
Chains of length 3, see data online for other motifs

Each entry in the table has two values. Both are log<sub>10</sub>(p-value).

1. The first (left) value is the empirical p-value using Monte-Carlo activity-shuffling.
2. The second (right) value is obtained by fitting a gamma distribution to the set of values obtained using shuffling (see figure 2 in main text, and method section).

| Condition         | Tchn3:FwdOnOff  | Tchn3:FwdOffOn  | Tchn3:BwdOnOff  | Tchn3:BwdOffOn  |
|-------------------|-----------------|-----------------|-----------------|-----------------|
| sorbitol          | 1.04,0.96,n= 38 | 0.15,0.15,n= 26 | 0.26,0.29,n= 15 | 0.11,0.11,n= 24 |
| diamide           | 1.84,2.07,n=113 | 0.04,0.08,n= 6  | 0.70,0.68,n= 57 | 0.15,0.20,n= 12 |
| menadiione        | 0.31,0.52,n= 7  | 0.17,0.17,n= 26 | 0.19,0.40,n= 5  | 0.36,0.34,n= 38 |
| 25C               | 0.59,0.67,n= 10 | 1.80,2.01,n=144 | 0.36,0.53,n= 7  | 0.82,0.83,n= 87 |
| 2.5 mM DTT        | 1.37,1.23,n= 9  | 0.01,0.01,n= 14 | 1.44,1.23,n= 10 | 0.13,0.13,n= 37 |
| Adenine starve    | 0.20,0.19,n= 41 | 0.13,0.36,n= 4  | 0.08,0.09,n= 31 | 0.22,0.40,n= 6  |
| Hypo-Osmotic      | 0.58,0.55,n= 33 | 0.25,0.30,n= 13 | 0.08,0.09,n= 14 | 1.50,1.46,n= 56 |
| N depletion       | 0.13,0.17,n= 11 | 0.82,0.83,n= 11 | 0.01,0.04,n= 4  | 0.57,0.71,n= 8  |
| H2O2              | 0.26,0.29,n= 16 | 0.63,0.61,n= 71 | 0.72,0.67,n= 32 | 0.53,0.52,n= 67 |
| dtt               | 0.38,0.37,n= 39 | 0.32,0.34,n= 20 | 1.14,1.19,n= 81 | 0.54,0.51,n= 28 |
| heat shock        | 3.85,4.47,n=268 | 0.14,0.27,n= 7  | 1.51,1.66,n=142 | 0.13,0.25,n= 7  |
| DES459 MMS        | 0.12,0.27,n= 5  | 0.17,0.23,n= 12 | 0.62,0.60,n= 18 | 0.50,0.49,n= 24 |
| DES460 MMS        | 0.66,0.65,n= 16 | 0.04,0.33,n= 1  | 0.45,0.49,n= 13 | 0.06,0.32,n= 2  |
| mecl+gamma        | 0.28,0.27,n= 35 | 0.01,0.02,n= 3  | 0.09,0.10,n= 23 | 1.48,1.55,n= 70 |
| wt+gamma          | 1.43,1.53,n=100 | 0.07,0.18,n= 5  | 0.21,0.20,n= 34 | 0.11,0.19,n= 7  |
| dioxidic shift    | 0.19,0.63,n= 2  | 0.21,0.57,n= 3  | 1.14,1.01,n= 14 | 0.18,0.51,n= 3  |
| SD                | 0.24,0.24,n= 46 | 0.94,0.83,n= 28 | 0.36,0.34,n= 58 | 0.55,0.53,n= 20 |
| SD+aa             | 0.35,0.36,n= 18 | 0.04,0.07,n= 6  | 0.96,0.93,n= 41 | 0.31,0.32,n= 20 |
| SD+AR+I           | 0.13,0.14,n= 22 | 0.62,0.59,n= 24 | 0.46,0.45,n= 44 | 0.58,0.56,n= 25 |
| SD+LI+I           | 0.58,0.56,n= 57 | 0.10,0.37,n= 3  | 0.01,0.02,n= 13 | 0.41,0.53,n= 9  |
| SD+I              | 0.16,0.16,n= 35 | 0.77,0.69,n= 18 | 0.58,0.57,n= 68 | 0.26,0.36,n= 9  |
| SEtOH             | 0.53,0.92,n= 3  | 0.00,0.00,n= 0  | 3.00,1.80,n= 39 | 0.00,0.00,n= 0  |
| SEtOH+aa          | 0.81,1.01,n= 5  | 0.00,0.00,n= 0  | 5.00,3.33,n=139 | 0.00,0.00,n= 0  |
| SEtOH+aa+I        | 0.74,0.96,n= 5  | 0.00,0.00,n= 0  | 4.52,2.31,n= 74 | 0.37,0.85,n= 2  |
| SEtOH+I           | 1.37,1.18,n= 11 | 0.00,0.00,n= 0  | 3.46,2.00,n= 53 | 0.64,0.98,n= 3  |
| Gal               | 2.19,1.45,n= 30 | 0.00,0.00,n= 0  | 3.22,1.89,n= 58 | 0.00,0.00,n= 0  |
| Gal+aa            | 1.57,1.34,n= 8  | 0.32,0.80,n= 2  | 1.78,1.43,n= 13 | 0.29,0.76,n= 2  |
| Gal+aa+I          | 0.35,0.75,n= 3  | 0.26,0.84,n= 1  | 1.10,1.02,n= 11 | 0.23,0.80,n= 1  |
| Gal+I             | 0.27,0.65,n= 3  | 1.62,1.26,n= 13 | 1.63,1.19,n= 24 | 0.28,0.75,n= 2  |
| Acid              | 0.59,0.57,n= 37 | 1.43,1.28,n= 51 | 0.31,0.31,n= 27 | 0.31,0.34,n= 16 |
| Alkali            | 0.14,0.22,n= 9  | 1.03,0.99,n= 55 | 0.86,0.81,n= 34 | 0.15,0.16,n= 17 |
| Heat              | 1.48,1.65,n=134 | 0.00,0.00,n= 0  | 0.30,0.29,n= 54 | 0.79,0.85,n= 9  |
| NaCl              | 0.06,0.23,n= 3  | 0.38,0.38,n= 24 | 0.78,0.71,n= 20 | 0.10,0.13,n= 12 |
| Peroxide          | 0.52,0.72,n= 7  | 0.10,0.10,n= 46 | 0.27,0.58,n= 4  | 0.01,0.02,n= 26 |
| Sorbitol          | 0.36,0.44,n= 12 | 0.07,0.08,n= 26 | 1.71,1.36,n= 50 | 0.20,0.19,n= 41 |
| WT 0.0625M        | 2.05,1.34,n= 38 | 0.05,0.12,n= 5  | 2.00,1.39,n= 43 | 0.02,0.05,n= 4  |
| WT 0.125M         | 0.12,0.50,n= 2  | 0.04,0.05,n= 17 | 0.48,0.65,n= 7  | 0.08,0.09,n= 23 |
| WT 0.25M          | 0.70,0.93,n= 5  | 0.02,0.03,n= 23 | 1.59,1.23,n= 17 | 0.28,0.26,n= 58 |
| WT 0.5M           | 0.40,0.75,n= 4  | 0.17,0.16,n= 58 | 1.46,1.16,n= 19 | 0.14,0.14,n= 56 |
| WT 1M             | 0.84,0.84,n= 12 | 0.34,0.33,n= 62 | 0.79,0.81,n= 12 | 0.05,0.06,n= 32 |
| WT 1Msorb         | 0.22,0.52,n= 4  | 0.40,0.38,n= 74 | 1.21,0.97,n= 21 | 0.24,0.24,n= 62 |
| WT alpha          | 0.30,0.77,n= 2  | 0.35,0.51,n= 8  | 1.51,1.20,n= 16 | 0.62,0.61,n= 15 |
| hog1 0.125MK      | 1.05,0.88,n= 26 | 0.02,0.08,n= 3  | 2.44,1.95,n= 72 | 0.01,0.04,n= 2  |
| hog1 0.5M         | 0.50,0.69,n= 7  | 0.80,0.79,n= 81 | 0.24,0.54,n= 4  | 0.29,0.28,n= 49 |
| pbs2 0.5M         | 1.39,1.15,n= 12 | 0.01,0.03,n= 2  | 0.71,0.89,n= 6  | 0.17,0.22,n= 12 |
| shol 0.5M         | 1.25,1.08,n= 14 | 0.08,0.08,n= 37 | 2.11,1.41,n= 32 | 0.03,0.04,n= 30 |
| ssk1 0.0625M      | 0.45,1.01,n= 1  | 2.72,1.71,n= 60 | 0.37,0.94,n= 1  | 0.41,0.50,n= 10 |
| ssk1 0.125M       | 1.58,1.16,n= 35 | 0.07,0.14,n= 6  | 3.80,3.08,n=124 | 0.09,0.15,n= 8  |
| ssk1 0.5M         | 0.78,0.90,n= 8  | 0.01,0.02,n= 22 | 1.64,1.23,n= 23 | 0.02,0.02,n= 24 |
| ssk1shol 0.5M     | 0.58,0.81,n= 6  | 0.23,0.22,n= 49 | 1.43,1.14,n= 20 | 0.18,0.18,n= 47 |
| ssk1stell 0.0625M | 0.77,0.81,n= 11 | 1.35,1.20,n= 9  | 0.08,0.37,n= 2  | 0.63,0.92,n= 4  |
| ssk1stell 0.125M  | 0.00,0.00,n= 0  | 2.22,1.69,n= 59 | 0.00,0.00,n= 0  | 1.18,1.06,n= 37 |
| ssk1stell 0.25M   | 1.24,1.05,n= 14 | 0.24,0.24,n= 23 | 0.34,0.67,n= 4  | 0.21,0.21,n= 23 |
| ssk1stell 0.5M    | 0.87,0.84,n= 13 | 0.93,0.94,n= 74 | 0.45,0.62,n= 8  | 0.17,0.17,n= 32 |
| ssk1stell 1M      | 0.42,0.75,n= 4  | 0.01,0.02,n= 23 | 1.25,1.07,n= 15 | 0.06,0.06,n= 35 |
| stell 0.0625M     | 2.70,1.95,n= 75 | 0.00,0.00,n= 0  | 0.43,0.48,n= 13 | 0.00,0.00,n= 0  |
| stell 0.125M      | 0.00,0.00,n= 0  | 0.12,0.13,n= 32 | 2.07,1.39,n= 28 | 0.20,0.20,n= 42 |
| stell 0.5M        | 0.63,0.88,n= 5  | 0.04,0.04,n= 28 | 1.08,1.03,n= 10 | 0.16,0.15,n= 47 |
| WT Gal N2 R1      | 4.10,4.66,n=174 | 0.09,0.13,n= 9  | 0.45,0.44,n= 28 | 0.02,0.05,n= 5  |
| WT Gal N2 R2      | 2.52,2.62,n=104 | 0.04,0.06,n= 7  | 0.55,0.53,n= 30 | 0.14,0.16,n= 14 |
| WT Gal N2 R3      | 2.77,3.19,n=127 | 0.12,0.19,n= 9  | 0.97,0.96,n= 49 | 0.05,0.10,n= 6  |
| WT Glu N2 R1      | 1.16,1.00,n= 37 | 0.44,0.48,n= 14 | 0.20,0.26,n= 11 | 2.11,1.75,n= 65 |
| WT Glu N2 R2      | 0.32,0.33,n= 20 | 0.10,0.24,n= 5  | 0.09,0.12,n= 11 | 0.91,0.80,n= 27 |
| WT Glu N2 R3      | 0.93,0.81,n= 27 | 0.05,0.15,n= 5  | 0.53,0.53,n= 18 | 0.66,0.62,n= 25 |
| msn2/4 Gal R1     | 5.00,6.95,n=254 | 0.25,0.33,n= 11 | 2.15,2.40,n=105 | 0.13,0.22,n= 8  |
| msn2/4 Gal R2     | 4.00,4.75,n=177 | 0.02,0.14,n= 2  | 0.69,0.65,n= 33 | 0.02,0.12,n= 2  |
| msn2/4 Gal R3     | 5.00,3.84,n=176 | 0.09,0.16,n= 7  | 1.02,0.86,n= 26 | 0.05,0.11,n= 6  |
| Mean WT Gal       | 3.15,3.56,n=135 | 0.07,0.12,n= 8  | 0.57,0.54,n= 31 | 0.03,0.07,n= 6  |
| Mean WT Glu       | 1.27,1.06,n= 38 | 0.01,0.13,n= 1  | 0.00,0.00,n= 0  | 0.95,0.82,n= 25 |
| Mean msn2/4       | 4.70,5.88,n=218 | 0.14,0.29,n= 6  | 1.25,1.20,n= 51 | 0.15,0.28,n= 7  |
| Zakrzewska        | 0.46,0.45,n= 25 | 0.06,0.43,n= 1  | 2.73,3.31,n=127 | 0.00,0.00,n= 0  |
| 18733             | 0.24,0.33,n= 11 | 0.39,0.44,n= 15 | 0.36,0.39,n= 15 | 0.40,0.43,n= 16 |
| 18734             | 0.04,0.09,n= 5  | 0.91,0.84,n= 39 | 0.16,0.20,n= 11 | 0.69,0.65,n= 32 |
| 18735             | 0.31,0.36,n= 15 | 0.16,0.33,n= 6  | 0.69,0.64,n= 27 | 0.41,0.50,n= 12 |
| 6035              | 0.39,0.53,n= 9  | 0.30,0.29,n= 29 | 0.30,0.46,n= 8  | 0.63,0.62,n= 47 |
| hdf1              | 0.10,0.14,n= 9  | 0.92,0.88,n= 44 | 0.15,0.19,n= 12 | 0.09,0.11,n= 12 |

**Table S4: list of timed enzymes (see additional lists online)**

| Reaction name           | orfname | protein |
|-------------------------|---------|---------|
| YJR159W_SOT>FRU         | YJR159W | SOR1    |
| YBR299W_MLT>GLC         | YBR299W | MAL32   |
| YGR287C_MLT>GLC         | YGR287C | YGR287C |
| YGR292W_MLT>GLC         | YGR292W | MAL12   |
| YIL172C_MLT>GLC         | YIL172C | YIL172C |
| YJL216C_MLT>GLC         | YJL216C | YJL216C |
| YJL221C_MLT>GLC         | YJL221C | FSP2    |
| YJL153C_G6P>MI1P        | YJL153C | INO1    |
| YHR046C_MI1P>MYOI       | YHR046C | YHR046C |
| YGR060W_O2+MZYMS>IZYMST | YGR060W | ERG25   |
| YGL001C_IZYMST>IIZYMST  | YGL001C | ERG26   |
| YLR100W_IIZYMST>ZYMST   | YLR100W | ERG27   |
| YOL061W_R5P>PRPP        | YOL061W | PRS5    |
| YBL068W_R5P>PRPP        | YBL068W | PRS4    |
| YER099C_R5P>PRPP        | YER099C | PRS2    |
| YHL011C_R5P>PRPP        | YHL011C | PRS3    |
| YKL181W_R5P>PRPP        | YKL181W | PRS1    |
| YJL005W_ATP>cAMP        | YJL005W | CYR1    |
| YLR028C_IMP>PRFICA      | YLR028C | ADE16   |
| YLR359W_ASUC>FUM        | YLR359W | ADE13   |
| YLR359W_FUM>ASUC        | YLR359W | ADE13   |
| YAR073W_IMP>XMP         | YAR073W | IMD1    |
| YHR216W_IMP>XMP         | YHR216W | IMD2    |
| YML056C_IMP>XMP         | YML056C | IMD4    |
| YLR432W_IMP>XMP         | YLR432W | IMD3    |
| YAR075W_IMP>XMP         | YAR075W | YAR075W |
| YML035C_AMP>IMP         | YML035C | AMD1    |
| YGL248W_cAMP>AMP        | YGL248W | PDE1    |
| YOR360C_cAMP>AMP        | YOR360C | PDE2    |
| YEL021W_OMP>UMP         | YEL021W | URA3    |
| YKL024C_UMP>UDP         | YKL024C | URA6    |
| YNR012W_UR1>UMP         | YNR012W | URK1    |
| YJR103W_UTP>CTP         | YJR103W | URA8    |
| YKL067W_UDP>UTP         | YKL067W | YKL067W |
| YGR194C_XUL>X5P         | YGR194C | XKS1    |
| YLR027C_OA>ASP+AKG      | YLR027C | AAT2    |
| YLR027C_AMP>AKG+OA      | YLR027C | AAT2    |
| YDR111C_PYR>AKG+ALA     | YDR111C | YDR111C |
| YDR111C_AKG+ALA>PYR     | YDR111C | YDR111C |
| YPR145W_AMP>GLN>ASN     | YPR145W | ASN1    |
| YGR124W_AMP>GLN>ASN     | YGR124W | ASN2    |
| YLR155C_ASN>ASP         | YLR155C | ASP3-1  |
| YLR160C_ASN>ASP         | YLR160C | ASP3-4  |
| YDR321W_ASN>ASP         | YDR321W | ASP1    |
| YER055C_PPRPP>PPRBATP   | YER055C | HIS1    |
| YDR127W_SME5P+PEP>3PSME | YDR127W | ARO1    |
| YGL148W_3PSME>CHOR      | YGL148W | ARO2    |
| YPR060C_CHOR>PHEN       | YPR060C | ARO7    |
| YNL316C_PHEN>PHPYR      | YNL316C | PHA2    |
| YHR137W_PHPYR>AKG+PHE   | YHR137W | ARO9    |
| YBR166C_PHEN>4HPP       | YBR166C | TYR1    |
| YKL106W_4HPP>AKG+TYR    | YKL106W | AAT1    |
| YDR261C_13GLUCAN>GLC    | YDR261C | EXG2    |
| YGR282C_13GLUCAN>GLC    | YGR282C | BGL2    |
| YLR300W_13GLUCAN>GLC    | YLR300W | EXG1    |
| YOR190W_13GLUCAN>GLC    | YOR190W | SPR1    |
| YCL025C_ASNxt>ASN       | YCL025C | AGP1    |
| YDR508C_ASNxt>ASN       | YDR508C | GNP1    |
| YPL265W_ASNxt>ASN       | YPL265W | DIP5    |
| YFL055W_SERxt>SER       | YFL055W | AGP3    |
| YCL025C_SERxt>SER       | YCL025C | AGP1    |
| YDR508C_SERxt>SER       | YDR508C | GNP1    |
| YKR039W_SERxt>SER       | YKR039W | GAP1    |
| YPL265W_SERxt>SER       | YPL265W | DIP5    |
| YBR069C_THR>THRxt       | YBR069C | TAT1    |
| YCL025C_THR>THRxt       | YCL025C | AGP1    |
| YDR508C_THR>THRxt       | YDR508C | GNP1    |
| YBL042C_UR1xt>UR1       | YBL042C | FUI1    |
| YKL217W_PYRxt>PYR       | YKL217W | JEN1    |
| YOR011W_ZYMST>ZYMSTxt   | YOR011W | AUS1    |

## Table S5: Properties of Timed Enzymes

### A. Flexibility:

#### All Genes

Genes in ordered motifs: 0.73+-0.70 (n=76)  
The rest: 0.08+-0.18 (n=520)  
T-test p-value (ttest) = 0.000000, -log10(p) (ttest) = 56.128  
Wilcoxon test p-value (Wilcoxon) = 0.000000, -log10(p)(Wilcoxon)= 16.551  
Linear Correlation r=0.6162, p=0.000000, -log10(p)(Corrcoef)= 62.871  
Spearman Rank-Corr r=0.4147, t=11.1075, p=0.000000, -log10(p)(Spearman)= Inf  
T-test (high/low T=1.00) t=11.1075, p=0.000000, -log10(p)(ttest2)= 64.428

#### Metabolic

Genes in ordered motifs: 0.73+-0.70 (n=76)  
The rest: 0.09+-0.19 (n=424)  
T-test p-value (ttest) = 0.000000, -log10(p) (ttest) = 46.558  
Wilcoxon test p-value (Wilcoxon) = 0.000000, -log10(p)(Wilcoxon)= 15.589  
Linear Correlation r=0.6133, p=0.000000, -log10(p)(Corrcoef)= 52.253  
Spearman Rank-Corr r=0.4039, t=9.8534, p=0.000000, -log10(p)(Spearman)= Inf  
T-test (high/low T=1.00) t=9.8534, p=0.000000, -log10(p)(ttest2)= 52.636

#### Metabolic Enzymes

Genes in ordered motifs: 0.63+-0.72 (n=61)  
The rest: 0.06+-0.13 (n=373)  
T-test p-value (ttest) = 0.000000, -log10(p) (ttest) = 36.350  
Wilcoxon test p-value (Wilcoxon) = 0.000000, -log10(p)(Wilcoxon)= 11.507  
Linear Correlation r=0.6069, p=0.000000, -log10(p)(Corrcoef)= 44.303  
Spearman Rank-Corr r=0.3433, t=7.5964, p=0.000000, -log10(p)(Spearman)= 12.721  
T-test (high/low T=1.00) t=7.5964, p=0.000000, -log10(p)(ttest2)= 48.860

#### Metabolic Transporters

Genes in ordered motifs: 1.12+-0.43 (n=15)  
The rest: 0.24+-0.38 (n=51)  
T-test p-value (ttest) = 0.000000, -log10(p) (ttest) = 9.914  
Wilcoxon test p-value (Wilcoxon) = 0.000005, -log10(p)(Wilcoxon)= 5.338  
Linear Correlation r=0.8096, p=0.000000, -log10(p)(Corrcoef)= 15.722  
Spearman Rank-Corr r=0.7165, t=8.2167, p=0.000000, -log10(p)(Spearman)= 10.873  
T-test (high/low T=1.00) t=8.2167, p=0.000000, -log10(p)(ttest2)= 14.256

#### CCM Enzymes

Genes in ordered motifs: 0.47+-0.65 (n=30)  
The rest: 0.09+-0.21 (n=59)  
T-test p-value (ttest) = 0.000082, -log10(p) (ttest) = 4.088  
Wilcoxon test p-value (Wilcoxon) = 0.000578, -log10(p)(Wilcoxon)= 3.238  
Linear Correlation r=0.4834, p=0.000002, -log10(p)(Corrcoef)= 5.796  
Spearman Rank-Corr r=0.3141, t=3.0862, p=0.002719, -log10(p)(Spearman)= 2.566  
T-test (high/low T=1.00) t=3.0862, p=0.000001, -log10(p)(ttest2)= 5.891

#### CCM Transporters

Genes in ordered motifs: 0.15+-0.22 (n=2)  
The rest: 0.29+-0.44 (n=16)  
T-test p-value (ttest) = 0.685940, -log10(p) (ttest) = 0.164  
Wilcoxon test p-value (Wilcoxon) = 0.836601, -log10(p)(Wilcoxon)= 0.077  
Linear Correlation r=0.2075, p=0.408686, -log10(p)(Corrcoef)= 0.389  
Spearman Rank-Corr r=0.3220, t=1.3604, p=0.192571, -log10(p)(Spearman)= 0.715  
T-test (high/low T=1.00) t=1.3604, p=0.300783, -log10(p)(ttest2)= 0.522

### CCM Enzymes & Transporters

Genes in ordered motifs: 0.45+-0.63 (n=32)  
The rest: 0.13+-0.28 (n=75)  
T-test p-value (ttest) = 0.000472, -log10(p) (ttest) = 3.326  
Wilcoxon test p-value (Wilcoxon) = 0.003135, -log10(p)(Wilcoxon)= 2.504  
Linear Correlation r=0.4366, p=0.000003, -log10(p)(Corrcoef)= 5.587  
Spearman Rank-Corr r=0.2983, t=3.2022, p=0.001806, -log10(p)(Spearman)= 2.743  
T-test (high/low T=1.00) t=3.2022, p=0.000002, -log10(p)(ttest2)= 5.759

### CCM+1 Enzymes

Genes in ordered motifs: 0.74+-0.70 (n=75)  
The rest: 0.11+-0.22 (n=235)  
T-test p-value (ttest) = 0.000000, -log10(p) (ttest) = 26.955  
Wilcoxon test p-value (Wilcoxon) = 0.000000, -log10(p)(Wilcoxon)= 11.554  
Linear Correlation r=0.5952, p=0.000000, -log10(p)(Corrcoef)= 30.371  
Spearman Rank-Corr r=0.4093, t=7.8729, p=0.000000, -log10(p)(Spearman)= 13.224  
T-test (high/low T=1.00) t=7.8729, p=0.000000, -log10(p)(ttest2)= 29.583

### CCM+1 Transporters

Genes in ordered motifs: 0.86+-0.66 (n=38)  
The rest: 0.15+-0.31 (n=100)  
T-test p-value (ttest) = 0.000000, -log10(p) (ttest) = 13.715  
Wilcoxon test p-value (Wilcoxon) = 0.000000, -log10(p)(Wilcoxon)= 7.189  
Linear Correlation r=0.6844, p=0.000000, -log10(p)(Corrcoef)= 19.668  
Spearman Rank-Corr r=0.4906, t=6.5659, p=0.000000, -log10(p)(Spearman)= 8.997  
T-test (high/low T=1.00) t=6.5659, p=0.000000, -log10(p)(ttest2)= 20.077

### CCM+1 Enzymes & Transporters

Genes in ordered motifs: 0.74+-0.70 (n=75)  
The rest: 0.11+-0.23 (n=256)  
T-test p-value (ttest) = 0.000000, -log10(p) (ttest) = 28.220  
Wilcoxon test p-value (Wilcoxon) = 0.000000, -log10(p)(Wilcoxon)= 11.847  
Linear Correlation r=0.5897, p=0.000000, -log10(p)(Corrcoef)= 31.664  
Spearman Rank-Corr r=0.4106, t=8.1676, p=0.000000, -log10(p)(Spearman)= 14.162  
T-test (high/low T=1.00) t=8.1676, p=0.000000, -log10(p)(ttest2)= 32.227

## B. Protein Noise:

### All Genes

Genes in ordered motifs: 3.41+-5.63 (n=64)  
The rest: 0.65+-3.32 (n=1977)  
T-test p-value (ttest) = 0.000000, -log10(p) (ttest) = 9.682  
Wilcoxon test p-value (Wilcoxon) = 0.000001, -log10(p)(Wilcoxon)= 5.830  
Linear Correlation r=0.1159, p=0.000000, -log10(p)(Corrcoef)= 6.704  
Spearman Rank-Corr r=0.4795, t=24.4440, p=0.000000, -log10(p)(Spearman)= Inf

### Metabolic

Genes in ordered motifs: 3.41+-5.63 (n=64)  
The rest: 1.90+-3.94 (n=226)  
T-test p-value (ttest) = 0.014815, -log10(p) (ttest) = 1.829  
Wilcoxon test p-value (Wilcoxon) = 0.093937, -log10(p)(Wilcoxon)= 1.027  
Linear Correlation r=0.1061, p=0.072327, -log10(p)(Corrcoef)= 1.141  
Spearman Rank-Corr r=0.2411, t=4.2014, p=0.000035, -log10(p)(Spearman)= 4.450

### Metabolic Enzymes

Genes in ordered motifs: 2.87+-4.99 (n=56)  
The rest: 1.78+-3.77 (n=209)  
T-test p-value (ttest) = 0.075526, -log10(p) (ttest) = 1.122

Wilcoxon test p-value (Wilcoxon) = 0.251159,  $-\log_{10}(p)$ (Wilcoxon)= 0.600  
Linear Correlation r=0.0708, p=0.250810,  $-\log_{10}(p)$ (Corrcoef)= 0.601  
Spearman Rank-Corr r=0.2225, t=3.7010, p=0.000262,  $-\log_{10}(p)$ (Spearman)= 3.582

### Metabolic Transporters

Genes in ordered motifs: 7.20+-8.40 (n=8)  
The rest: 3.15+-5.79 (n=15)  
T-test p-value (ttest) = 0.186637,  $-\log_{10}(p)$  (ttest) = 0.729  
Wilcoxon test p-value (Wilcoxon) = 0.185746,  $-\log_{10}(p)$ (Wilcoxon)= 0.731  
Linear Correlation r=0.4341, p=0.038477,  $-\log_{10}(p)$ (Corrcoef)= 1.415  
Spearman Rank-Corr r=0.4276, t=2.1678, p=0.041814,  $-\log_{10}(p)$ (Spearman)= 1.379

### CCM Enzymes

Genes in ordered motifs: 2.52+-4.61 (n=33)  
The rest: 2.87+-4.51 (n=35)  
T-test p-value (ttest) = 0.748899,  $-\log_{10}(p)$  (ttest) = 0.126  
Wilcoxon test p-value (Wilcoxon) = 0.515462,  $-\log_{10}(p)$ (Wilcoxon)= 0.288  
Linear Correlation r=-0.1373, p=0.264203,  $-\log_{10}(p)$ (Corrcoef)= 0.578  
Spearman Rank-Corr r=-0.0589, t=-0.4796, p=0.633063,  $-\log_{10}(p)$ (Spearman)= 0.199

### CCM Transporters

Genes in ordered motifs: 2.70+-3.65 (n=2)  
The rest: 2.49+-2.71 (n=3)  
T-test p-value (ttest) = 0.945638,  $-\log_{10}(p)$  (ttest) = 0.024  
Wilcoxon test p-value (Wilcoxon) = 0.800000,  $-\log_{10}(p)$ (Wilcoxon)= 0.097  
Linear Correlation r=0.3612, p=0.550319,  $-\log_{10}(p)$ (Corrcoef)= 0.259  
Spearman Rank-Corr r=0.0000, t=0.0000, p=1.000000,  $-\log_{10}(p)$ (Spearman)= -0.000

### CCM Enzymes & Transporters

Genes in ordered motifs: 2.53+-4.51 (n=35)  
The rest: 2.84+-4.37 (n=38)  
T-test p-value (ttest) = 0.762832,  $-\log_{10}(p)$  (ttest) = 0.118  
Wilcoxon test p-value (Wilcoxon) = 0.497084,  $-\log_{10}(p)$ (Wilcoxon)= 0.304  
Linear Correlation r=-0.1286, p=0.278304,  $-\log_{10}(p)$ (Corrcoef)= 0.555  
Spearman Rank-Corr r=-0.0482, t=-0.4066, p=0.685517,  $-\log_{10}(p)$ (Spearman)= 0.164

### CCM+1 Enzymes

Genes in ordered motifs: 3.52+-5.68 (n=62)  
The rest: 2.05+-4.13 (n=117)  
T-test p-value (ttest) = 0.048517,  $-\log_{10}(p)$  (ttest) = 1.314  
Wilcoxon test p-value (Wilcoxon) = 0.141890,  $-\log_{10}(p)$ (Wilcoxon)= 0.848  
Linear Correlation r=0.0946, p=0.207763,  $-\log_{10}(p)$ (Corrcoef)= 0.682  
Spearman Rank-Corr r=0.1651, t=2.2275, p=0.027177,  $-\log_{10}(p)$ (Spearman)= 1.566

### CCM+1 Transporters

Genes in ordered motifs: 5.60+-6.67 (n=20)  
The rest: 2.60+-3.86 (n=41)  
T-test p-value (ttest) = 0.029425,  $-\log_{10}(p)$  (ttest) = 1.531  
Wilcoxon test p-value (Wilcoxon) = 0.092512,  $-\log_{10}(p)$ (Wilcoxon)= 1.034  
Linear Correlation r=0.2379, p=0.064829,  $-\log_{10}(p)$ (Corrcoef)= 1.188  
Spearman Rank-Corr r=0.2485, t=1.9706, p=0.053464,  $-\log_{10}(p)$ (Spearman)= 1.272

### CCM+1 Enzymes & Transporters

Genes in ordered motifs: 3.52+-5.68 (n=62)  
The rest: 2.07+-4.04 (n=124)  
T-test p-value (ttest) = 0.046483,  $-\log_{10}(p)$  (ttest) = 1.333  
Wilcoxon test p-value (Wilcoxon) = 0.178665,  $-\log_{10}(p)$ (Wilcoxon)= 0.748  
Linear Correlation r=0.0941, p=0.201561,  $-\log_{10}(p)$ (Corrcoef)= 0.696  
Spearman Rank-Corr r=0.1621, t=2.2279, p=0.027102,  $-\log_{10}(p)$ (Spearman)= 1.567

**Table S6: Pairs of matching transcription factors and conditions**

|                                    |   |
|------------------------------------|---|
| Adenine starvation<br>(Gasch 2000) | ADR1 SM, ARG80 SM, ARG81 SM, ARO80 SM, BAS1 SM, CAD1 SM, CBF1 SM, CHA4 SM, DAL81 SM, DAL82 SM, FHL1 SM, GAT1 SM, GCN4 SM, GCR2 SM, GLN3 SM, HAP4 SM, HAP5 SM, LEU3 SM, MET28 SM, MET31 SM, MET32 SM, UGA3 SM, MET4 SM, MOT3 SM, PHO2 SM, PUT3 SM, RAP1 SM, STP1 SM, RCS1 SM, RPH1 SM, RTG1 SM, RTG3 SM, SFP1 SM, SIP4 SM. |
| Heat shock<br>(Gasch 2000)         | ADR1 HEAT, GAT1 HEAT, HSF1 HEAT, MSN2 HEAT, SKN7 HEAT, YAP1 HEAT.   |
| Heat<br>(Causton 2001)             | ADR1 HEAT, GAT1 HEAT, HSF1 HEAT, MSN2 HEAT, SKN7 HEAT, YAP1 HEAT  |
| Acid<br>(Causton 2001)             | MSN2 Acid, MSN4 Acid  |